

Applicants: Holloway et al.

Serial No.: Unknown (Parent: 09/606,031)

Docket No.: 99-29C1

For: SECRETED PROTEIN ZACRP4

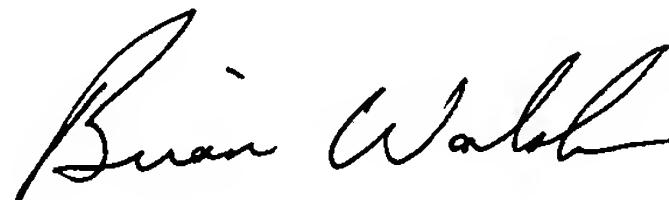
REMARKS

The Examiner is respectfully requested to consider and to enter the above amendments. The specification has been amended to correct certain grammatical and typographical errors.

Summary

If for any reason the Examiner believes that a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (206) 442-6540.

Respectfully Submitted,



Brian J. Walsh
Registration No. 45,543

Enclosures:

Express Mail Certificate
Filing Under 37 C.F.R. §1.53(b) (in duplicate)
Patent Application
Figure
Unexecuted Combined Declaration and Power of Attorney
Sequence Listing
ASCII Computer Disk Sequence pursuant to 37 CFR 1.821(f)
Preliminary Amendment with accompanying Appendix A
Petition and Fee for Extension of Time (in duplicate)
Postcard

APPENDIX A – SPECIFICATION AMENDMENTS WITH NOTATIONS TO
INDICATE CHANGES MADE

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Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 6, line 32, has been amended as follows:

The Figure illustrates an alignment of the aromatic motifs within the first and second C1q domains of zacrp4. C1q1 corresponds to amino acid residues 50-134 of SEQ ID NO:2, and C1q2 corresponds to amino acid residues 203-286 of SEQ ID NO:2. The aromatic motif within the first C1q domain (C1q1) is from amino acid residue 50 to amino acid residue 80 of SEQ ID NO:2. The aromatic motif within the second C1q domain (C1q2) is from amino acid residue 203 to amino acid residue 233 of SEQ ID NO:2.

The paragraph beginning at page 8, line 5, has been amended as follows:

The term “contig” denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to “overlap” a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' (SEQ ID NO:10) are 5'-TAGCTTgagtct-3' (SEQ ID NO:11) and 3'-gtcgacTACCGA-5' (SEQ ID NO:12).

The paragraph beginning at page 15, line 1, has been amended as follows:

TABLE [3] 2

Amino Acid	One-Letter Code	Codons	Degenerate Codon
Cys	C	TGC, TGT	TGY
Ser	S	AGC, AGT, TCA, TCC, TCG, TCT	WSN
Thr	T	ACA, ACC, ACG, ACT	CAN
Pro	P	CCA, CCC, CCG, CCT	CCN
Ala	A	GCA, GCC, GCG, GCT	GCN
Gly	G	GGA, GGC, GGG, GGT	GGN
Asn	N	AAC, AAT	AAY
Asp	D	GAC, GAT	GAY
Glu	E	GAA, GAG	GAR
Gln	Q	CAA, CAG	CAR
His	H	CAC, CAT	CAY
Arg	R	AGA, AGG, CGA, CGC, CGG, CGT	MGN
Lys	K	AAA, AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA, ATC, ATT	ATH
Leu	L	CTA, CTC, CTG, CTT, TTA, TTG	YTN
Val	V	GTA, GTC, GTG, GTT	GTN
Phe	F	TTC, TTT	TTY
Tyr	Y	TAC, TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA, TAG, TGA	TRR
Asn Asp	B		RAY
Glu Gln	Z		SAR
Any	X		NNN
Gap	-	---	

The paragraph beginning at page 28, line 13, has been amended as follows:

For any zacrp4 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 herein. Moreover, those of skill in the art can use standard software to devise zacrp4 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of the following sequences: SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO: [10] 4. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (*e.g.*, CD-read only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (*e.g.*, DVD-ROM, DVD-RAM, and DVD+RW).

The paragraph beginning at page 56, line 10, has been amended as follows:

It is possible that an improper remodeling response to connective tissue or muscle injury in the joints results in sensitivity to excessive release of cellular components at the site of the injury. Zacrp[6]4 polypeptides, fragments, fusions and the like would be useful in determining if an association exists between such a response and the inflammation associated with arthritis. Such indicators include a reduction in inflammation and relief of pain or stiffness. In animal models, indications would be derived from macroscopic inspection of joints and change in swelling of hind paws. Zacrp[6]4 polypeptides, fragments, fusions and the like can be administered to animal models of osteoarthritis (Kikuchi et al., Osteoarthritis Cartilage 6:177-86, 1998 and Lohmander et al., Arthritis Rheum. 42:534-44, 1999) to look for inhibition of tissue destruction that results from inflammation stimulated by the action of collagenase.

The paragraph beginning at page 63, line 8, has been amended as follows:

Novel zacrp4 polynucleotides and polypeptides of the present invention were initially identified querying an expressed sequence tag (EST) database for proteins having homology to adipocyte complement related proteins. To identify the corresponding cDNA sequence, a clone was isolated from an arrayed human pituitary cDNA/plasmid library. The library was screened by PCR using oligonucleotides ZC20,839 (SEQ ID NO:5) and ZC20,840 (SEQ ID NO:6). Thermocycler conditions were as follows: 1 cycle at 94°C for 2 minutes 30 seconds, followed by 30 cycles at 94°C for 10 seconds, 64°C for 20 seconds, 72°C for 30 seconds, ending with a 7 minute extension at 72°C. The library was deconvoluted down to a positive pool of 250 clones. *E. coli* **ElectroMAX[®]** DH10B cells (GIBCO BRL, Gaithersburg, MD) were transformed with this pool by electroporation following manufacturer's protocol. The transformed culture was titered and arrayed into a 96 well plate at ~20 cells/well. The cells were grown overnight at 37°C in LB + ampicillin. An aliquot of the cells were pelleted and screened using PCR to identify positive wells using oligonucleotide primers and PCR conditions were as described above. The remaining cells in the positive wells were plated and colonies screened by PCR to identify a single positive clone. The clone was subjected to sequence analysis using a Hybaid OmniGene Temperature Cycling System (National Labnet Co., Woodbridge, NY). Sequencher[®] 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. The resulting 1196 bp sequence is disclosed in SEQ ID NO:1.

The paragraph beginning at page 63, line 32, has been amended as follows:

Northern blots were performed using Human Multiple Tissue Blots **(MTNTM)** (MTN1, MTN2 and MTN3) from Clontech (Palo Alto, CA) were probed to determine the tissue distribution of human zacrp4. A clone described above was used as a template for the generation of a 303 bp cDNA probe based on the initially discovered EST sequence (SEQ ID NO:7) using the PCR. Oligo nucleotides ZC20,839 (SEQ ID NO:5) and ZC20,840 (SEQ ID NO:6) were used as primers. The

probe was purified using a Gel Extraction Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions. The probe was then radioactively labeled using a Rediprime II DNA labeling kit (Amersham, Arlington Heights, IL) according to the manufacturer's specifications. The probe was purified using a NUCTrap® push column (Stratagene Cloning Systems, La Jolla, CA). ExpressHyb™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 55°C, using 1.5×10^6 cpm/ml labeled probe. The blots were then washed in 2X SSC and 0.1% SDS at room temperature, then with 2X SSC and 0.1% SDS at 65°C, followed by a wash in 0.1X SSC and 0.1% SDS at 65°C. A single transcript of approximately 1.4 kb was seen in brain, spinal cord, ovary, and testis. Fainter signals were detected in thyroid, adrenal gland, bone marrow, small intestine, prostate, liver and colon.

The paragraph beginning at page 64, line 12, has been amended as follows:

A RNA Master Dot Blot™ (Clontech) that contained RNAs from various tissues that were normalized to 8 housekeeping genes were also probed and hybridized as described above. Expression was seen in all brain tissues and in fetal brain, spinal cord, ovary and pituitary gland. Fainter signals were detected in testis, uterus, prostate, salivary gland, lymph node and fetal liver and lung.

The paragraph beginning at page 64, line 30, has been amended as follows:

For the mapping of zacrp4 with the GeneBridge 4 RH Panel, 20 µl reactions were set up in a 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2 µl 10X KlenTaq PCR reaction buffer (Clontech Laboratories, Inc., Palo Alto, CA), 1.6 µl dNTPs mix (2.5 mM each, Perkin-Elmer, Foster City, CA), 1 µl sense primer, ZC 22,162, (SEQ ID NO:8), 1 µl antisense primer, ZC 22,168 (SEQ ID NO:9), 2 µl RediLoad (Research Genetics, Inc.), 0.4 µl 50X Advantage® KlenTaq Polymerase Mix (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and ddH₂O for a total volume of 20 µl. The

Appendix A

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reactions were overlaid with an equal amount of mineral oil and sealed. The PCR
cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 94°C, 40
cycles of a 45 seconds denaturation at 94°C, 45 seconds annealing at 66°C and 1
minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7
minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel
(Life Technologies, Gaithersburg, MD).

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